SOME EFFECTS OF X-RADIATION ON THE NEUROBLAST CHROMOSOMES OF THE GRASSHOPPER, CHORTOPHAGA VIRIDIFASCIATA

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RECENT studies and interpretations of the genetic effects of X-ray induced translocations and inversions in plants and animals emphasize the need for additional information concerning the cytological aspects of such chromosome alterations. Realization of this has prompted a number of investigators (LEWITSKY and ARARATIAN 1931; MATHER and STONE 1933; STONE 1933; MATHER 1934, 1937; HUSKINS and HUNTER 1935; WHITE 1935, 1937; RILEY 1936; LEVAN 1936; GUSTAFSSON 1937; and NEBEL 1936, 1937) to undertake studies of the immediate effects of X-rays on chromosome morphology. Because the results of these studies, though agreeing in many essentials, nevertheless seem utterly opposed and contradictory in other respects, further work is needed, utilizing other and, as far as possible, more favorable material.

The neuroblasts of the embryo of the grasshopper, *Chortophaga viridifasciata*, were used in the present study. These cells are particularly suitable for observations of chromosome form and alteration (CARLSON 1937). They are present in relatively large numbers. They are readily identifiable because of their large size, which is sufficient to accommodate the metaphase and anaphase chromosomes with a minimum of crowding and overlapping; this is especially important in X-rayed material, in which one must be able to distinguish between translocation and juxtaposition. Also, like the better-known germ cell chromosomes of the grasshopper, these somatic chromosomes are large. In this species all are telomitic, and so have the form of simple rods. On the other hand, a disadvantage of this material, especially as compared with the microspores of certain plants, is the large number of chromosomes (23 and 24 in the male and female, respectively), which makes it impossible to identify and interpret changes in terms of individual chromosomes, after even a relatively slight X-ray treatment.

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MATERIAL AND METHODS

Eggs containing embryos 3–5 weeks old were given X-ray dosages of 100, 125, 250, 500, 750, and 1000 r.[1] At the end of certain time intervals after irradiation embryos were removed from the eggs and made into permanent slides by BAUER'S (1936) modification of the aceto-carmine method. This consists of immersion for 30–40 minutes in aceto-carmine, spreading on an albuminized glass slip under a greased cover glass, removal of the cover glass in 95 percent alcohol, and mounting directly in euparal.

It is true that fixation and staining with aceto-carmine do not give the uniformly and consistently excellent chromosome preparations that many other technics do. The ease and speed that it affords, however, recommend its use where a large number of preparations must be made in a limited period of time, provided, of course, that the shortcomings of the method be kept in mind in interpreting results. The aceto-carmine method described above gives preparations that are adequate for the purposes of the present study.

EFFECTS ON MITOSIS

In the normally developing embryo at any given time different neuroblasts are in different stages of mitosis. Following irradiation there is a rapid disappearance of middle prophase through telophase stages. At the end of one and a half hours, after 500 r, only interphases and early prophases are left. This cessation of mitosis persists for a period of time varying with the X-ray dosage. Anaphases first begin to reappear after about 3, 7, 17, and 22 hours following dosages of 100, 250, 750, and 1000 r, respectively. Additional data on this effect will be included in a later paper.

The present study is based primarily on material fixed within a few hours of the end of the period of cessation, in order to obtain stages of the same mitotic cycle in which irradiation occurred. Since the mitotic cycle of the neuroblast requires at least two days, and probably often more, for its completion, alterations dealt with in the present paper are solely immediate effects.

CHROMOSOME ALTERATIONS

The findings of several investigators support the view that X-radiation may alter the chromosomes at any stage of the mitotic cycle. These changes are much more drastic, however, when the chromatin is diffuse, that is, in telophase through early prophase, than in stages in which it is

[1] In view of the recent conclusion of Gustafsson (1936, 1937) that in plant root tips the frequency of chromosome X-ray effects increases with the increase in water content of the tissues, it seems important to remark that all eggs used in the present study were kept saturated with water for at least several days previous to treatment.

concentrated in well-formed late prophase, metaphase, and anaphase chromosomes. MATHER and STONE (1933), WHITE (1935), RILEY (1936), and GUSTAFSSON (1936, 1937) found alterations in cells irradiated at interphase and early prophase stages. In addition RILEY reported breaks produced in late prophase and metaphase chromosomes. HUSKINS and HUNTER (1935) described chromosome and chromatid fragmentation and translocation in cells treated at telophase.

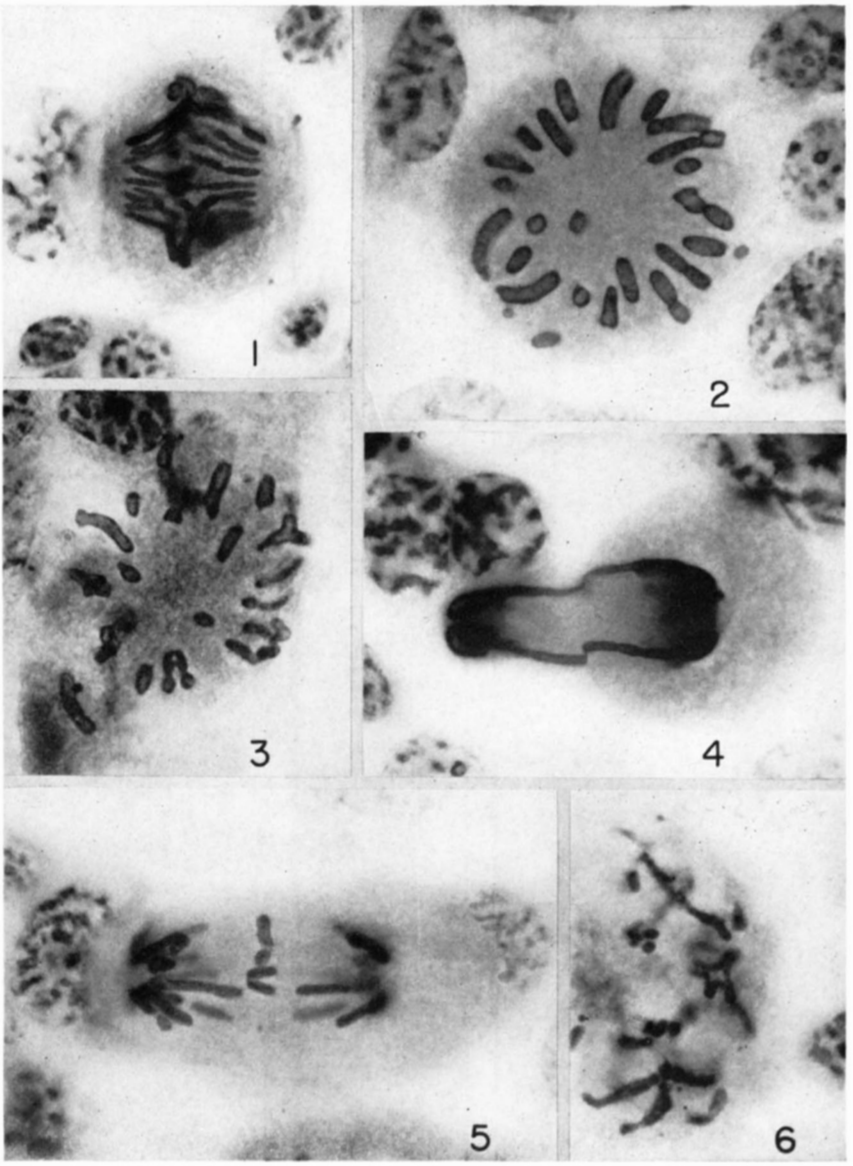
The chromosomes of mitotically active neuroblasts fixed within a few minutes after irradiation differ to some extent from normal, untreated ones. In late prophase sister chromatids appear to be very closely approximated at intervals along their length, these places having the appearance of constrictions. Metaphase chromosomes are longer than normal, exhibit severe constrictions, and show considerable twisting of sister chromatids about each other. Anaphase chromosomes appear joined together distally, while proximally they are elongated and show unusually broad constrictions (fig. 1).

X-ray effects that have occurred between telophase and mid-prophase, as observed in cells fixed in the subsequent metaphase and anaphase stages, show not only chromosome alterations but also chromatid and what may be half-chromatid inequalities. No evidence of either increase or decrease in the number of spindle fiber attachments was found in any of the cells in which accurate counts could be made.

In the following pages the term *fragment* is applied to a portion of a chromosome resulting from one or more breaks in an original chromosome and having no spindle fiber attachment. Either unaltered chromosomes or altered ones that have at least one spindle fiber attachment are referred to as *chromosomes*.

1. *Chromosome Fragmentation*

The fragments resulting from chromosome breakage are observable in the metaphase immediately following X-radiation. They are distinguishable from chromosomes with spindle fiber attachments, which are arranged with their kinetochores in an even circle and their distal ends extending outward, because they typically lie outside these chromosomes and have a less regular orientation (fig. 2). At early anaphase the "chromatids" of fragments, though lacking spindle attachments, separate at the same time as those of the chromosomes, which possess kinetochores. This separation may be complete or incomplete, but its occurrence proves conclusively that the initial anaphase separation of chromatids is entirely independent of the kinetochore (CARLSON 1938) and is probably autonomous. A more complete account of the behavior of fragments during mitosis is now in course of preparation.



FIGURES 1–6 —Photographs of irradiated neuroblast chromosomes. Length of time after irradiation: figure 1—about 3 min.; figure 5—12 hrs.; figures 3, 6—23 hrs.; figure 2—25 hrs.; figure 4—166 hrs. Dosage: figures 2, 4, 5—250 r; figure 1—500 r; figures 3, 6—750 r. ×1100.

FIGURE 1.—Anaphase. Pronounced constrictions present immediately after irradiation.

FIGURES 2, 3.—Metaphases. Fragments at periphery of cells. Note translocations in figure 3, one of which is shown in figure 16.

FIGURE 4.—Anaphase. Very long translocated chromosomes.

FIGURE 5.—Anaphase. Two V-shaped fragments in equatorial plane.

FIGURE 6 —Late prophase. Chromatid translocation. For details see figure 8.

The number of fragments per cell varies with the X-ray dosage. After 125 r there are rarely more than three or four fragments per cell, and frequently none at all. On the other hand, after 1000 r there may be more than thirty fragments per cell and no cells lacking them entirely. Because fusion of fragments *inter se* and with chromosomes obviously occurs with much frequency, counts of independent fragments give no accurate information of the actual amount of breakage originally effected by treatment, and so were not attempted.

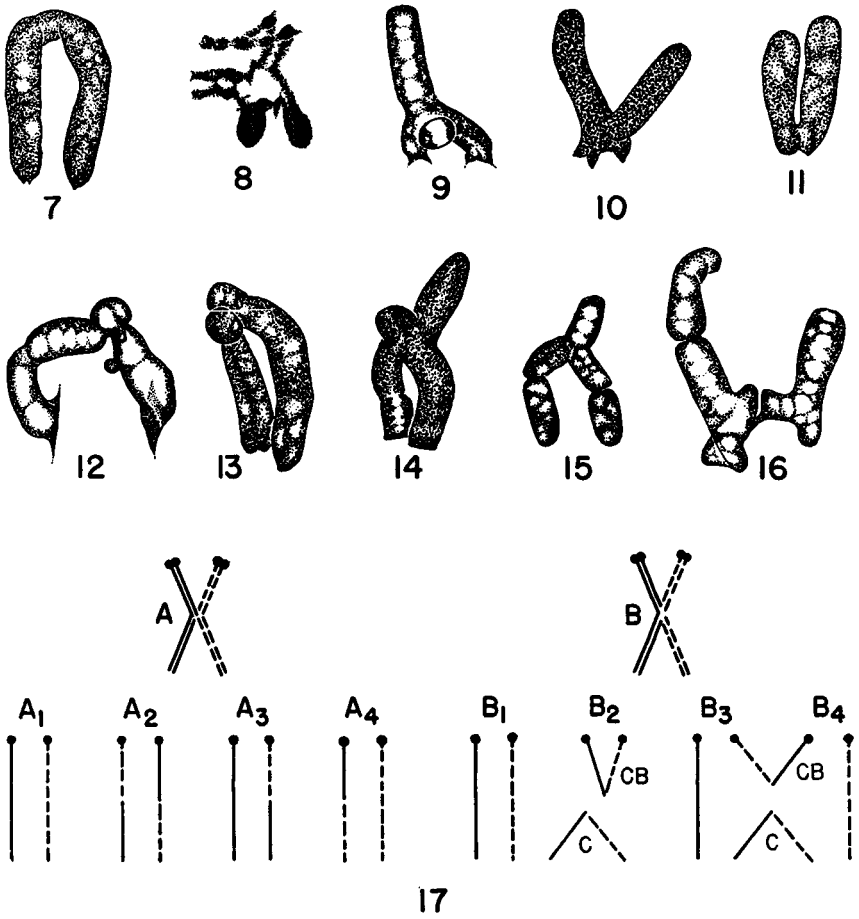
2. Chromosome Translocation

The large number of chromosomes per cell and lack of morphologically distinguishing characters make impossible any attempt to determine the number and extent of the chromosome translocations involved after irradiation.

Positive proof of translocation exists in the form of abnormally long chromosomes (fig. 4) and U-shaped chromosomes with two or more kinetochores (fig. 7). The anaphase behavior of the latter depends on the orientation of the kinetochores on the spindle (MATHER and STONE 1933; HUSTED 1936). If both spindle fiber loci of fused chromatids are directed toward the same pole, and the chromatids are not twisted about each other, a U-shaped daughter chromosome will move toward each pole (figs. 22, 23). If there is a half-turn in the chromosome, however, so that the spindle fiber loci of each fused chromatid point toward opposite poles, the anaphase configuration will have the form of two crossed chromatin bridges (figs. 24, 25). U-shaped chromosomes may persist as such, therefore, from one cell generation to the next, until they become oriented on the spindle with a half-turn from end to end. A delayed effect, resulting from the breakage of the crossed chromatin bridges and the subsequent formation of new attachments at the broken ends, might appear, as a consequence, a considerable number of cell generations after irradiation. The ultimate fate of chromatin bridges is considered in detail in a later section.

3. Chromatid Inequalities

Neuroblast chromosomes fixed and stained by most methods have, at metaphase and anaphase, a homogeneous appearance that affords no evidence of internal structure. In aceto-carmine smears, on the other hand, chromosomes in these stages usually exhibit a lightly staining "matrix" containing darker-staining regions comparable to the chromatids of the prophase (compare figs. 8 and 9). The metaphase chromosome and fragment each contains two such parallel regions (figs. 2, 3, 9, 12, 16, 26, 27). The anaphase chromosome and fragment each shows one—it may be double—lying in the middle of the chromosome and extending from end



FIGURES 7–17.—Irradiated chromosomes with more than one spindle attachment as a result of translocations. Spindle attachment ends, except in figure 17, are directed toward bottom of page. Figure 8 is in late prophase; others in metaphase. Length of time after irradiation: figures 13, 14—10 hrs.; figures 7, 15—12 hrs.; figures 11, 12—18 hrs.; figures 8, 9, 10, 16—23 hrs. Dosage: figures 1, 7, 8, 9—250 r; figure 5—500 r; figures 2, 3, 4, 6, 10—750 r. ×2475.

FIGURE 7.—Chromosome translocation.

FIGURES 8–16.—Chromatid translocations. Figures 8 and 16 appear as photographs in figures 6 and 3, respectively.

FIGURE 17.—Diagram of chromosomes with different types of chromatid translocations, showing the possible distribution of their chromatids at anaphase. A (diagram of figure 9) will give combinations A_1 and A_2 or A_3 and A_4 at anaphase. B (diagram of figures 6 and 8) will give combinations B_1 and B_2 or B_3 and B_4. CB, chromatin bridge. C, "chromatid" lacking spindle attachment. Knobs at ends of chromosomes and chromatids indicate positions of spindle attachments.

to end (figs. 5, 18, 28–31, 33–37). Although the appearance of these regions suggests that they are tightly coiled chromonemata, it seems best, until they have been examined after other treatment, to refer to them and the "matrix" around them non-committally as chromatids.

HUSKINS and HUNTER (1935) have demonstrated that some of the chromosomal constrictions so numerous in irradiated material are actually chromatid breaks. Certain X-ray induced constrictions present in my material appear to be the result of incomplete fusions of chromosomes, the ends of one chromatid of each chromosome having united and the other chromatids having remained unattached. A fragment of this type appears in figure 37. In the ensuing anaphase the distal portion of the interrupted chromatids will constitute a chromatid fragment (fig. 18 A). The non-corresponding constrictions exhibited by the daughter chromosome pairs shown in figures 18 A, 19, and 21 appear to be chromatid inequalities, but may possibly represent half-chromatid effects.

A frequently observed type of chromatid inequality in my material is what may be called a chromatid translocation (figs. 3, 8–17), namely, the "lateral translocation" of HUSKINS and HUNTER (1935), "reciprocal chromatid fusion" of WHITE (1935), or "pseudobivalent" of LEVAN (1937). The fusion may give either of two configurations. (1) If the proximal portion of one chromatid of each of the two chromosomes involved is joined to the distal portion of the other chromosome, each of the four chromatids will have a single kinetochore (figs. 9, 17 A), and the configuration will resemble the cross-shaped diakinetic tetrad. At anaphase two chromatids will pass to each pole, the orientation of the kinetochores on the spindle determining whether both (fig. 17, A_1 and A_2) or neither (A_3 and A_4) of the daughter cells will get a complete complement of chromosomes. (2) If the two proximal and the two distal portions are joined *inter se* at the point of breakage, the chromosome will consist of two unaltered chromatids, one chromatid with two kinetochores, and one with none (figs. 6, 8, 17 B). If the unaltered chromatids pass to the same pole (fig. 17 B_1), the one with the two kinetochores will pass to the other pole (B_2); but if the former pass to opposite poles (B_3 and B_4), the kinetochores of the latter will also move toward opposite poles, thus forming a chromatin bridge (CB). In each case the "chromatid" without a kinetochore (C) may pass into either of the daughter cells. Only one of these four possible daughter cells will possess a complete set of chromosomes, namely, the one into which the two unaltered chromatids pass.

4. Half-chromatid Inequalities

NEBEL and RUTTLE have concluded from observations of the somatic chromosomes of Tradescantia (1935, 1936) and Hordeum, Secale, and Crocus (1937) that doubling of the chromonemata occurs two generations in advance of their anaphase separation, so that from one anaphase through the next prophase each chromatid is composed of two chromonemata or half-chromatids. I have found a few figures in my prepara-

tions that seem very suggestive of half-chromatid effects, though none furnishes what I consider to be entirely convincing proof of the occurrence of such inequalities. This is due in part to the nature of this problem, itself, and in part to the fact that I have looked for these only in stages of the division immediately following irradiation, while the presence or absence of half-chromatid effects should be more readily detectable in the second division following irradiation.

In figures 18 A, 19, and 21 are shown anaphase pairs of daughter chromosomes that were irradiated in the interphase or early prophase condition. They exhibit constrictions at non-corresponding places. That such constrictions are not comparable to the secondary constrictions normally present in untreated material of many different organisms is evident from the fact that, in the latter, sister chromatids are constricted at corresponding points. They may, however, be comparable to the constrictions in metaphase chromosomes resulting from a break in one of the two chromatids (HUSKINS and HUNTER 1935), but, because they occur in anaphase instead of metaphase chromosomes, they would represent, by analogy, half-chromatid breaks instead of chromatid breaks.

One member of each of pairs A and B (fig. 18) displays a short region of abnormally small diameter. Comparison of the lengths of each of these with its sister chromosome suggests that the segment of small diameter has been inserted, its small size being due, perhaps, to the possibility that it is an inserted half-chromatid.

The lower chromosome of pair A (fig. 18) and one "chromatid" of the fragment shown in figure 34 have small blobs of chromatin protruding from the side. Underneath each and corresponding to it closely in size is a lightly staining area. It seems not unlikely that each little blob may be a piece of a half-chromatid that has been "knocked out" of the central part of the chromatid, but has remained connected with it peripherally.

CHROMATIN BRIDGES AND FRAGMENTS

The fact that X-ray studies of Drosophila salivary gland chromosomes have as yet failed to disclose a single positive case of a terminal inversion has led many to hold that a broken end of a chromosome cannot exist as such, at least over the course of many cell generations, and that, therefore, if a single break occurs, it is somehow eliminated. It is generally assumed that some kind of unsatisfied attraction causes broken ends of chromosomes to unite with one another. In the cell shown in figure 2 an original set of 23 chromosomes with 46 ends has been altered by irradiation to give a total of 26 chromosomes and fragments possessing together 52 ends. This and many other similar examples leave no doubt that new ends, whether of chromosomes or fragments or both, may be formed as a result of X-radiation.

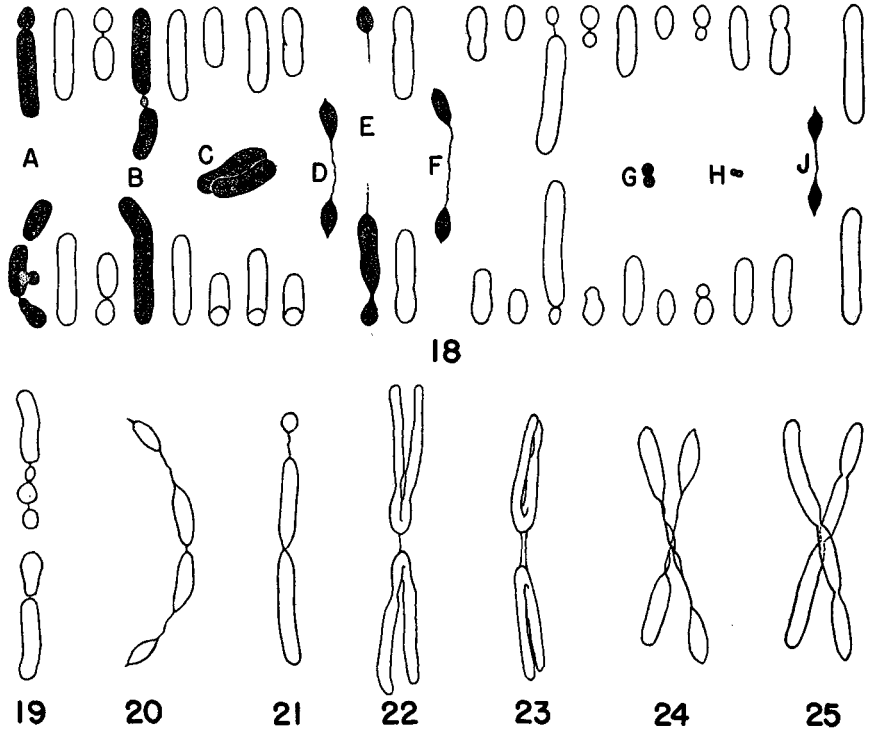
My material suggests, however, that the presence of such new ends does not actually demonstrate the existence of new, unsaturated ends of gene strings. It appears, instead, that in these instances the broken ends of sister chromatids of chromosomes and fragments have fused *inter se*. This assumption makes understandable two classes of chromosomal forms observed at anaphase, namely, chromatin bridges and fragments of three kinds.

A lagging at anaphase of certain daughter chromosomes and great attenuation of their distal ends to form chromatin bridges (fig. 18, D, F, J) result from failure of these ends to separate normally. It seems probable that the distal ends of the chromatids of such a chromosome are newly formed ends, which, unable to satisfy their attraction for broken ends of the chromatids of another chromosome, have fused with one another and tend to retain this union even at anaphase (fig. 38 CB).

The chromatin bridges shown in figure 18 (D, F, J) and in figure 20 may be expected to part at the site of the former break, so that equal daughter chromosomes with the same broken ends will pass into different daughter cells. If in the next cell generation the broken ends of the sister chromatids are again fused, chromatin bridges will be formed anew. Such broken ends can certainly, therefore, be transmitted from one cell generation to the next and may be supposed to persist in this manner from cell generation to cell generation until one of the following events occurs. (1) The broken end may unite with the broken end of a fragment at some subsequent cell generation to produce a normally behaving translocated chromosome (the "delayed attachment" of STADLER 1932). This presupposes that fragments may pass from parent to daughter cells, and this frequently occurs in these cells (CARLSON 1938). (2) The broken end may unite with the broken end of another chromosome to form a U-shaped chromosome with two spindle attachments. The mitotic behavior and probable ultimate fate of these has been described above. (3) The broken end may eventually acquire the properties of a true end, and so the chromatids cease to fuse at their broken ends to form chromatin bridges. Such chromosomes will then become normally behaving chromosomes with terminal deficiencies. This possibility is suggested by the X-chromosome deficiencies described by DEMEREC and HOOVER (1936) in Drosophila. (4) Sufficient genic material may be lost in the remnants of the chromatin bridge left outside the nucleus at the telophase of several succeeding divisions to produce a deficiency that is lethal to the cells. (5) The point of breakage of the chromatin bridge does not always occur at the same place, that is, at the point of chromatid fusion (fig. 18 E, 21), so that a deficiency is produced in one cell and a duplication in the other. Either or both may be cell lethal. The importance of any of these changes from the

genetic aspect will, of course, depend on whether or not the cell survives them; for, if it does not, the altered chromosomes will be lost with the cell.

Chromatin bridges have been observed in a number of different organisms in the first meiotic anaphase. They have been interpreted as chromatids with two spindle fiber loci resulting from crossing over in the inverted



FIGURES 18–25.—Anaphase separation of daughter chromosomes following irradiation. *Length of time after irradiation:* figures 19, 21—12 hrs.; figure 20—22 hrs.; figure 18—23 hrs.; figure 24—47 hrs.; figure 22—75 hrs.; figure 25—119 hrs.; figure 23—143 hrs. Dosage: figures 18, 19, 21–25—250 r; figure 20—500 r. ×1585.

FIGURE 18.—Complete set of anaphase chromosomes and fragments. A and B, chromatid or half-chromatid (?) inequalities. C, G, H, fragments. D, E, F, J, chromatin bridges.

FIGURE 19.—Chromatid or half-chromatid (?) inequality.

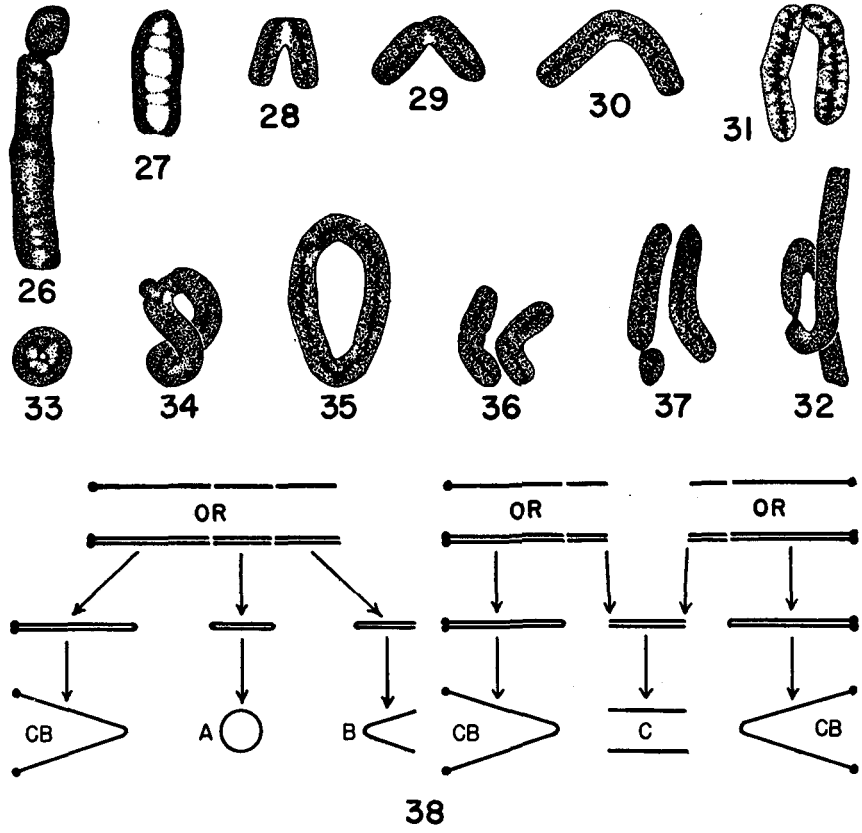
FIGURE 20.—Chromatin bridge.

FIGURE 21.—Chromatid or half-chromatid (?) inequality and chromatin bridge.

FIGURES 22–25.—Types of separation of chromosomes with two spindle attachments.

portion of chromosomes in inversion heterozygotes. In the microspores studied by HUSTED (1936) and in my material, however, another explanation has been necessary to account for these chromatin bridges, since synapsis and crossing over cannot have been involved. To sum up, the conclusion seems justifiable that the chromatin bridges in my material have arisen in three different ways: (1) from chromsome translocation,

(2) from chromatid translocation, and (3) from fusion of sister chromatids at their broken ends.



FIGURES 26–38.—Autonomous separation of "chromatids" of X-ray produced fragments, which lack spindle attachments. Length of time after irradiation: figures 27–32, 35, 36—12 hrs.; figure 26—13 hrs.; figures 33, 37—21 hrs.; figure 34—23 hrs. Dosage: figures 26–32, 35, 36—250 r; figures 33, 34, 37—750 r. ×2475.

FIGURE 26.—Metaphase fragment.

FIGURE 27.—Early anaphase. Beginning of separation of "chromatids" except at one end.

FIGURES 28–32.—Middle anaphase. V-shaped fragments resulting from "chromatid" separation except at one end. See figure 38 B.

FIGURES 33–35.—Anaphase. Ring-shaped fragments resulting from separation of "chromatids" except at both ends. See figure 38 A.

FIGURES 36, 37.—Anaphase. Complete separation of "chromatids" to give two rod-shaped fragments. See figure 38 C.

FIGURE 38.—Diagram showing types of breakage and fusion that lead to formation at anaphase of chromatin bridges (CB) and fragments having the form of V's (B), rings (A), and double rods (C). Knobs at ends of chromosomes and chromatids indicate location of spindle attachments.

During early anaphase the "chromatids" of fragments may behave in any of three different ways[2] (CARLSON 1938). First, they may separate

[2] This classification does not include the small spherical fragments that are present in most cells after treatment.

except at one end to form a V (figs. 5, 27–32). Second, they may remain connected at both ends and separate centrally to form a ring (figs. 33–35). Third, they may separate completely to form two rods lying side by side (figs. 36, 37). The fact that the "chromatids" of fragments, which lack kinetochores, begin to separate with the advent of anaphase, at the same time as the chromosomes with kinetochores, indicates that separation is an intrinsic character of the chromosome. Their tendency to remain attached frequently at either one or both ends, however, points not only to some fundamental difference in the ends as compared with the central parts of the fragments, but also to differences in the ends of the fragments. A given fragment end may be either the original end of the chromosome of which it was once a part, or a new one created in the breakage of the original chromosome at that point. The "chromatids" of a terminal fragment would be expected to fuse with one another at their broken ends. At anaphase these ends would remain fused, as the opposite true ends separated, to give a V-shaped fragment (fig. 38 B). Fusion would be expected to occur at both ends of an intercalary fragment, and the retention of this union after the central portion of the "chromatids" had separated would give the ring-shaped anaphase fragment (fig. 38 A). Finally, if a given fragment were the result of fusion at the broken ends of the terminal fragments of two original chromosomes, the remaining ends would be true ends, and so the "chromatids" would be expected to separate completely at anaphase to form two distinct rods (fig. 38 C). V's are the most abundant type. Next in frequency are the two rods. Rings occur only rarely at dosages below 500 r, but frequently at 750 and 1000 r.

In figure 18 three fragments (C, G, H) and four chromatin bridges (D, E, F, J) appear. According to the above hypothesis they would be explained thus. Fragments G and H are assumed to represent the true ends of two of the chromosomes connected by bridges. Fragment C is separating as two rod-shaped chromatid fragments. Both ends are, therefore, true ends, having resulted from the fusion, at their broken ends, of two distal fragments from the other two chromosomes with bridges.

UPCOTT (1937) and BARBER (1938) have reported chromatin bridges in microspores lacking fragments. The latter has shown, however, that aging of the pollen somehow causes a fusion of true ends of sister chromatids.

DISCUSSION

Time of chromosome doubling. The conclusions of different cytologists regarding the time of doubling, or splitting, of the chromosomes have recently been summarized by GUSTAFSSON (1936) and KAUFMANN (1936). Views range all the way from doubling in the late interphase or early pro-

phase immediately preceding anaphase to doubling in the prophase one mitotic cycle in advance of anaphase separation. Most of the chromosomes on which my results are based were probably in late interphase or early prophase at the time of X-radiation, and both chromosome and chromatid effects were produced. If the half-chromatid effects suggested by my material are valid, doubling has occurred in the late interphase or early prophase one mitotic cycle in advance of anaphase separation of the units thus formed. If one disregards these, however, there remains no important evidence bearing on this problem.

Mechanism of chromosomal change. According to the hypothesis proposed by SEREBROVSKY (1929) X-rays effect structural changes in the chromosomes by causing fusions of different chromosomes or different parts of a single chromosome, which is followed by breakage in a different plane. The proposal of STADLER (1932) is just the reverse of this; fusion is supposed to follow breakage rather than precede it. If it may be assumed that in either hypothesis the first occurrence conditions the second, an increase in the total number of chromosomal elements, namely, chromosomes and fragments, which is invariably the situation in cells showing any effects at all, seems to support the concept of breakage preceding fusion. In none of the affected cells in my material is there any decrease in the number of chromosomal elements, which would result if there were a predominance of fusion over breakage. Another difficulty of the SEREBROVSKY hypothesis is its dependence on close proximity or contacts between chromosomes at the time of effective irradiation, that is, between late telophase and early prophase. This requirement may be realized in many types of cells. The grasshopper neuroblast, however, is an unusually large cell, containing a large lobed nucleus with a central cytoplasmic core. The ends of three or four of the longer chromosomes extend distally into each of five or six lobes. Contacts between different chromosomes, therefore, are considerably limited. It seems improbable that a sufficient number of contacts could exist between these chromosomes at the time of irradiation to account for the complex fusion configurations present in many of these cells after treatment with 1000 r, unless it is assumed that treatment, itself, causes extreme movement of the chromosomes with the establishment of new contacts as treatment progresses. Of particular interest in this connection is a questionable translocation involving the X-chromosome and an autosome in figure 15 of WHITE'S 1935 paper on Locusta. The X chromosomes of many Acrididae, and, therefore, probably of Locusta, lie in vesicles apart from the other chromosomes from telophase through late prophase. The irradiation bringing about this translocation doubtless occurred during this period (this cell was fixed 17 hours after treatment),

and breakage could have occurred at that time. Fusion with the autosomal piece must have occurred subsequently, however, after the disappearance of the membrane of the vesicle at late prophase.

SUMMARY

The neuroblasts of grasshopper embryos treated with 100, 125, 250, 500, 750, and 1000 r suffer a cessation of mitosis for a period of time proportional to the dosage.

Cells irradiated between telophase and early prophase show, in succeeding stages of the same mitotic cycle, chromosome fragmentation and translocation, chromatid breakage and translocation, and what may be half-chromatid effects.

Chromatin bridges at anaphase appear to result from any of three different alterations: (1) chromosome translocation, (2) chromatid translocation, (3) fusion of sister chromatids of the proximal portions of fragmented chromosomes at their broken ends.

Chromatin bridge formation and the persistence of broken ends of chromosomes from one cell generation to another makes possible delayed reattachments following irradiation.

The early anaphase separation of chromatids, judged on the basis of the behavior of fragments lacking spindle attachments, is entirely independent of the kinetochore, and so is probably an autonomous function of the chromosome.

An hypothesis is suggested to account for three different forms, namely, V's, rings, and rods, which "chromatids" of these fragments assume as they begin to separate at early anaphase.

Certain evidence suggests that chromosomal change is effected by fusion following, rather than preceding, breakage.

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